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The Embryological Origin of Pigment Cells in Platyfish-Swordtail Hybrids[1]

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(Plates I & II; Text-figure 1)

Introduction

THE first evidence concerning the embryological origin of pigment cells in vertebrates was supplied by Borcea (1909) in observations made on the development of several species of marine fishes. He asserted that teleost chromatophores developed from ectodermal cells in the dorsal portion of the nervous system. Later, Weidenreich (1912) discussed the ontogeny and phylogeny of vertebrate pigmentation and suggested that a positive correlation existed between the central nervous system and the primary distribution of pigment cells in animals representing several vertebrate classes.

DuShane (1935), who worked with salamander embryos, was the first to demonstrate experimentally in any vertebrate that the primary pigmentation was derived from cells of the neural crest which arose in close association with the nervous system. Likewise Dorris (1938, 1939) showed, by transplanting neural crest tissue from chicken embryos of a black strain to embryos of a white strain, that feather pigmentation in these animals was produced by cells that arose from the neural crest. Ris (1941) extended this observation and showed that the neural crest cells were the only source of pigmentation in birds.

The embryology of pigment cells in fishes differs somewhat from those forms already mentioned, in that fishes do not possess a group of cells that arises as does the neural crest in the amphibian and the chick. In fishes the nervous system arises as a solid rod and becomes hollow by cavitation. The cells in fishes that would be analogous to the neural crest are found in and along the length of the dorsal portion of the nervous system. These cells might be expected to be determined as pro-pigment cells (or melanoblasts) during the formation of the nerve cord and to migrate at a later stage. They transform first into melanocytes and then melanophores.

Lopashov (1944) investigated the origin of pigment cells in the perch (*Perca*) and two species of loach, (*Misgurnus* and *Nemacheilus*). Pieces of nervous system, when transplanted under the yolk sac epithelium of another embryo, gave rise to pigment cells. In most cases mesoderm alone failed to produce pigment cells. Similarly Newth (1951), who investigated the derivatives of the neural tissue in the lamprey (*Lampetra planeri*), showed that melanophores arose from this source.

It has long been considered possible that the etiology of pigmented tumors in mammals might be traced back to neural crest cells. There is evidence indicative of this. For example, Rawles (1953) showed that the pigment cells of mice have a neural crest origin. Neural crest cells, at least that portion represented by the pro-pigment cells, are intensively migratory, moving to all portions of the embryos, along almost any cell surface. Similarly, the melanoma has been recognized as one of the most readily metastasizing atypical growths (Raven, 1953), and this was believed to be due to an inherent tendency of tumorous melanocytes to become detached, singly or in groups, and escape into the blood and lymph system. The capacity to develop melanin is another characteristic that has been recognized as being possessed in common by neural crest cells and melanoma cells. Since melanomas have been studied metabolically (Greenstein & Algire, 1944; Greenstein, Werne,

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Eschenbrenner & Lewthardt, 1944; Burke, Algire, Hesselbach, Fischer & Legallais, 1948; DuBuy, Woods, Burke & Lackey, 1949; Humm & Clark, 1955) with respect to enzyme activity patterns, direct evidence indicating the metabolic similarity between melanoma and neural crest cells would also be highly desirable. Technical difficulties, however, have stood in the way. A significant contribution to this problem has been made by Flickinger (1949), who studied the metabolism of the neural crest of salamanders and was able to observe tyrosinase activity in these cells.

An ideal approach to a study of the total etiology of the melanotic tumor would be achieved were it possible to characterize a unique type of cell in the tumor of an adult whose embryo lends itself to microsurgical technique, and to trace this cell type back to its site of origin in the embryo. Regrettably, no melanomas are known in urodeles or chickens, although one was reported in a salamander and then lost (Sheremetieva-Brunst & Brunst, 1948). Fortunately melanoma may be produced at will in platyfish-swordtail hybrids, and it has been shown by Gordon (1937) that the melanosis and subsequent melanoma are always associated with macromelanophores. These unique cells are easily identified.

Gordon, in a series of papers which he reviewed in 1951, showed that the black pigmentation of the platyfish, *Xiphophorus maculatus,* is the result of the distribution of two different types of melanophores. Gordon (1927) showed that the first, and by far the most abundant of the platyfish's pigment cells are the *micromelanophores,* which are fairly heavily pigmented, highly dendritic and almost round in shape, with a diameter up to 100μ. These cells appear to have little or nothing to do with the production of melanomas. Micromelanophores are under the control of an autosomal gene. The second type of melanophore found in *X. maculatus* is also heavily pigmented and highly dendritic, but differs from the first type in size. These *macromelanophores* range from 100 to 300μ. Their presence, distribution and pattern of growth in the fish have been shown by Gordon (1948) to be under the control of a series of sex-linked genes.

The background color of the wild-type swordtail, *Xiphophorus helleri,* as in *X. maculatus,* is produced by many micromelanophores.

If a hybrid is produced by mating a swordtail with a platyfish that carries the sex-linked gene, *Sd* (for macromelanophore-spotting of the dorsal fin), the genic balance controlling the growth and differentiation of macromelanophores in the dorsal fin of the hybrid is disturbed. The result is a heavy overgrowth of these large black cells in the hybrid. The eventual result of such hypertrophic macromelanophore growth in these fish, as recently retraced by Ermin & Gordon (1955), is the gradual appearance of a distinctly invasive pigmented tumor.

Since the macromelanophore must be present before a hybrid fish can develop a melanoma (Gordon, 1937), this cell type represents an identifiable and essential component of the tumor. Therefore the embryological origin of the *macromelanophores* becomes of particular importance in a study of the etiology of melanomas.

The purpose of this study is to determine whether or not the potentially tumor-producing macromelanophores arise in the embryo of these hybrid fish from the same embryonic anlage as do the normal pigment cells. If it were possible to show that these cells arise as derivatives of the anterior-dorsal nervous system, and that these cells give rise first to melanosis and then to melanoma, this would constitute clear-cut experimental support for the neural origin of melanoma in at least one type of vertebrate.

The present experiments were designed to test the effect of transplanting presumptive melanoma-producing tissue of platyfish-swordtail hybrids into swordtail hosts which, although possessing genetic growth intensifier factors, could not develop melanomas themselves. The experiments reported in this paper verify Lopashov (1944) as to the neural site of pigment cell production in teleost fishes, and they show that both micromelanophores and macromelanophores appear when the dorsal portion of the nerve cord of a hybrid fish is grafted into a swordtail embryo.

Materials and Methods

The fish used in these experiments were obtained through the courtesy of Dr. Myron Gordon from the Genetics Laboratory of the New York Aquarium, New York Zoological Society. Platyfish (*Xiphophorus maculatus*), bearing the dominant sex-linked gene (*Sd*), for the occurrence of macromelanophores in the dorsal fin, in the homozygous condition, were crossed with recessive wild-type swordtails (*Xiphophorus helleri*). The swordtail carries certain modifying genes which act as intensifiers of macromelanophore growth (Gordon, 1937). The F_1 offspring were all heterozygous for the gene *Sd,* and all of them showed considerable melanosis in the dorsal fin.

The heterozygous melanotic F_1 hybrids (*Sd*+) were then back-crossed to recessive wild-type swordtails (++) and as a result, 50% of the backcross offspring had the macromelanophore

Sd gene; half were normal, homozygous recessives and had micromelanophores only. Those bearing the *Sd* gene showed advanced melanosis as small adults, owing to the atypical growth of macromelanophores. Many of them died from the invasive effects of the melanoma before becoming mature. Since the platyfish, the swordtails and their hybrids all have large numbers of micromelanophores uniformly distributed on the body surface, all donor and host embryos would be expected to produce micromelanophores during their development.

In accord with observations made by Hopper (1943) and Tavolga (1949) on the embrology of platyfish, swordtails and their hybrids, the timing of the stage of the embryos used was accomplished at first by making the assumption that the new complement of eggs in the female is fertilized and begins development seven days after the last brood of young are born. This assumption was experimentally verified in this laboratory. The normal embryological stages described by Tavolga (1949) have been used as a guide in the selection of appropriate stages of donor and host embryos.

The donor embryos used were backcross offspring obtained from the mating of *Sd* platyfish-swordtail hybrids back to wild-type swordtails, as described above. The most frequently used embryo donor stage, Tavolga Stage 10, may be summarized as follows: The blastopore is still open, although becoming narrow, 2 to 4 somites have made their appearance, the extra-embryonic membranes have grown posteriorly and are approaching the otic capsule.

The swordtail embryos which were used as hosts in the experiments were selected at Stage 20 for several reasons. At this stage the embryo is large enough to work with easily, but it has not yet become too active. Accordingly, it is somewhat easier to prepare the graft site and introduce the tissue. Also, at this stage the normal host pigmentation is confined to the retinal pigment and a few plate-like pigment cells in the cranial region, apparently on the meninges, as illustrated by Gordon (1931, fig. 37). The typical pigment cells are quite small, bi- or tri-polar cells and are oriented in the direction of their migration to the rear. One receives the distinct impression that the melanocytes advance from the head region posteriorly, since a mechanical obstacle will often appreciably delay pigmentation on the caudal side of an obstruction. Because of this delay in movement along the anterior-posterior pathway, it is possible to detect the early-appearing pigment cells on the graft before the host's pigment cells become too abundant to obscure the site of the graft. (Plate I, Figs. 1 and 2).

The grafting was carried out in Columbia watch glasses containing sterile 2% agar in which a slight depression had been made to hold the embryos. Donor embryos were removed from the yolk and carefully divided into pieces about 0.8 cu. mm. In order to avoid confusion concerning the origin of the graft, each embryo fragment was placed in a separate watch glass until used.

Host embryos were removed from the ovary of a gravid female under sterile conditions, washed 6 times with 3× Niu's (1954) solution[2], decapsulated, washed twice more and left in 3× Niu's solution until used. Decapsulation or removal of the chorion in these embryos is quite simple. Apparently the capsule is osmotically active since, when an embryo is placed in 3× Niu solution for a very short time, the capsule lifts sufficiently from the yolk to permit the insertion of the points of well sharpened Dumont watch-maker's forceps. Grasping the membrane over the embryo with a second pair of forceps breaks the chorion and the embryo comes out of the membrane with considerable force.

The operation on the host embryo was carried out using glass needles. A notch was cut in the back in the region anterior to the dorsal fin. Every precaution was taken to avoid injuring the nervous system and the underlying notochord. As fast as possible after the host had been prepared, the donor tissue from a platyfish-swordtail hybrid was picked up on the point of the glass needle and laid on the wound. No bridges were needed since the donor tissue was usually somewhat sticky, and when the two areas were contacted, attachment was instantaneous and healing very rapid. The watch glass was usually set aside for 5 to 10 minutes so that healing was well advanced before the embryos were transferred, by means of a wide mouth pipette, to a Stender dish containing 3× Niu's solution.

Maintainance of the experimental animals proved to be difficult. The early xiphophorin embryo develops a heavily vascularized serosa with which, it is presumed, it receives oxygen and nutrient from the mother and through which it eliminates waste. Scrimshaw (1944a, b, 1945) has shown that the xiphophorin embryo does not lose weight during its development, indicating that it is truly viviparous. It evidently obtains materials from the maternal circulation that are at least equivalent in weight to what is

[2] Niu's Solution (3×): Solutions A and B mixed 1:1.

A		B	
NaCl	—10.2 gm.	Na_2HPO_4	—0.33 gm.
KCl	— 0.15	KH_2PO_4	—0.06
$Ca(NO_3)_2 . 4H_2O$	— 0.24		
$MgSO_4$	— 0.30		

lost through metabolic end-products. Moreover, the presence of the serosa makes the removed embryo particularly vulnerable to differences in osmotic pressure and to traces of deleterious substances in the medium.

It was found that 3× Niu's solution was isosmotic with the embryonic circulation, but survival of the embryos in sufficient numbers to make the xiphophorin fish embryo a good experimental animal was not achieved until "conditioned" tank water, that is, water in which adult fish had lived for a time, was substituted for the double glass-distilled water. The results of this substitution were truly dramatic. For example, in 3× Niu's solution in double glass-distilled water that had been sterilized by autoclaving, a mortality of 50% after 4 days and 100% before 10 days was the rule. In Niu's solution made with "conditioned" tank water and sterilized by Seitz filtration, the post-operative mortality fell to zero. More than 80% of the embryos were successfully hatched and grown into young fish.

Tavolga & Rugh (1947) suspected that the pericardial serosa had a nutritive and excretory function in addition to the respiratory function suggested by Turner (1937). With this in mind, the post-operative embryos were placed in a medium of Niu's solution to which had been added 25 mgm% of glucose, 25 mgm% of casein hydrolysate, and a 2% solution of embryonic extract. Penicillin (100 units/100ml.) and streptomycin (20 mgm./100 ml.) were added as antibacterial agents. The air above the nutrient medium was replaced by acid-washed oxygen. The results of the added nutrient were not spectacular, although they were sufficiently marked to warrant the inclusion of these additions as part of the standard treatment. Over a period of two weeks the embryos' development in glucose, casein hydrolysate and embryonic extract was accelerated about one to two days over untreated controls.

Hatched young fish were acclimatized to "conditioned" aquarium water by progressive dilutions of 3× Niu's solution. It was found advisable to delay acclimatization to tank water until the yolk sac had almost disappeared. Apparently the intact yolk sac and yolk were in equilibrium with a concentration of 3× Niu's solution, and any decrease in this concentration before their resorption resulted in the appearance of fluid in the sac.

As soon as the yolk material was closed over by the body wall, the small fish were transferred to a finger bowl containing tank water and fed *Paramecium* and *Daphnia* until they were large enough to accept *Enchytraeus* and prepared fish food .

Results

The results reported here are concerned with the embryonic source of micro- and macromelanophores in the potentially melanomatous platyfish-swordtail hybrid (Text-figure 1). The possible effect of any of the modifying growth-stimulating factors in the normal swordtail host tissues as well as the possibility of inducing melanoma development in the host fish as a result of grafting neural tissue fragments will be considered in subsequent publications.

Origin of Macromelanophores

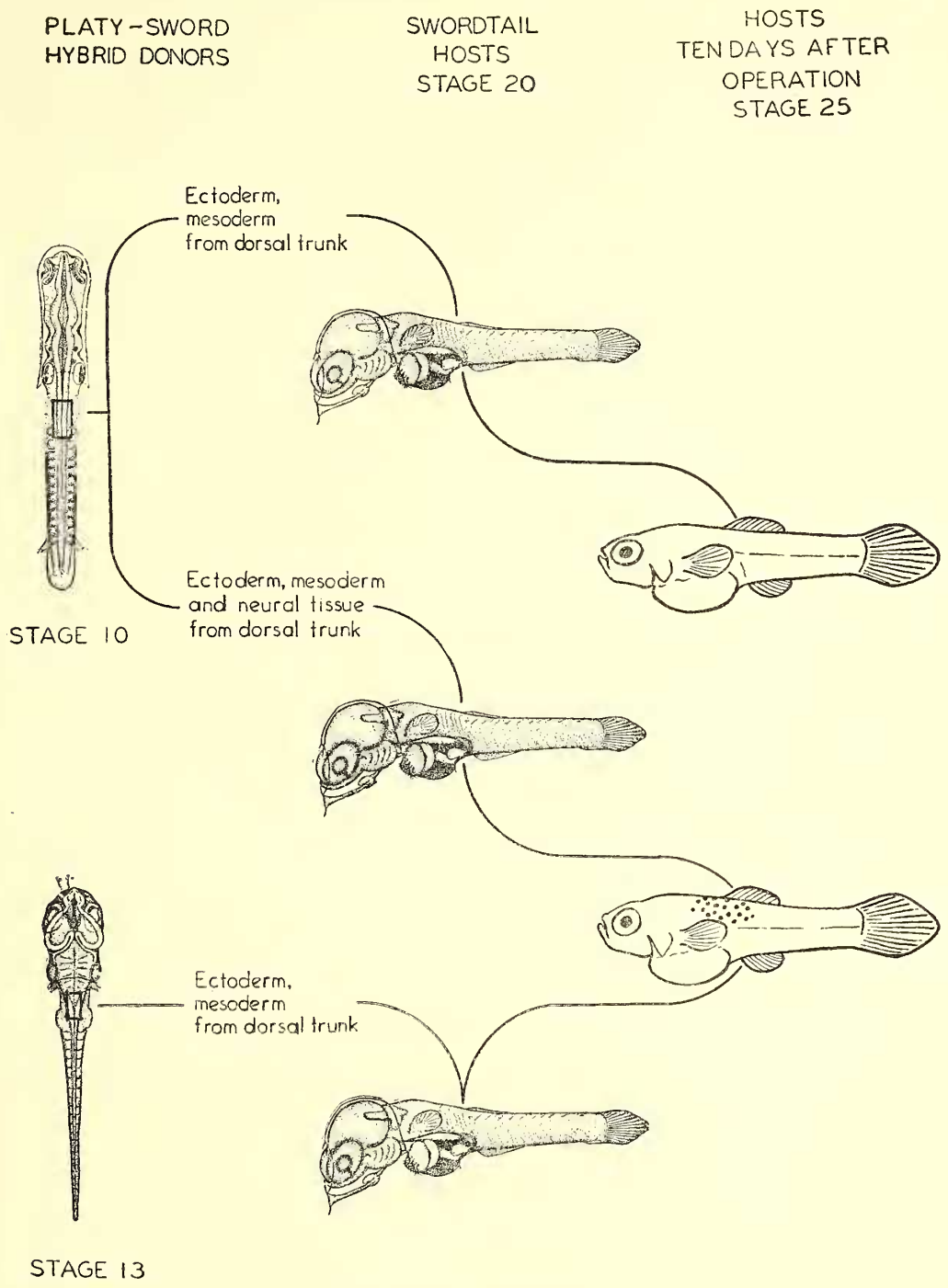
In two cases it was possible to demonstrate macromelanophores in the graft region of a host swordtail embryo (see Plate II, Figs. 3 & 4). The graft tissue had been taken from one *Sd* platyfish-swordtail hybrid from the trunk region just posterior to the hindbrain. From another *Sd* hybrid the tissue was taken from the same region just anterior to the dorsal fin. Both grafts from Stage 10 donors contained ectoderm, somite mesoderm and a portion of the nervous system.

The normal time for the appearance of macromelanophores in platyfish hybrids is considerably later than for the appearance of micromelanophores (Tavolga, 1949). Since at the time these experiments were performed, some difficulty was encountered in maintaining the embryos over long periods of time, many of the embryos unquestionably died before differentiation of macromelanophores could take place. Since macromelanophores do not occur in the genetic strain of swordtail employed by us, the presence of large pigment cells in these two swordtail embryo hosts indicates that the macromelanophores originated from the grafted tissue. They were definitely not normal host swordtail micromelanophores.

Origin of Micromelanophores

A word of explanation is required regarding the method used in making a decision for or against small pigment cell formation in a particular graft. The pigment cells of the swordtail host embryo appear first in the neck region, then rapidly populate the head and finally spread as an advancing wave down the trunk to cover the whole animal. Under the conditions used in these experiments, there was no way to distinguish directly between host and donor micromelanophores, except in those cases where the donor tissue also differentiated into macromelanophores. Accordingly the following criteria for identfication of donor melanophores were established and adhered to:

1. If, prior to the appearance of any host melanophores in the region of the graft, mel-



TEXT-FIG. 1. Diagrammatic summary of the grafting experiments showing the effect of the age of donor tissue and the germ layers used in the production of melanophores. (Drawings of embryo after Tavolga). Donor tissue from various regions of Stage 10 hybrid embryos was isolated by means of glass needles and transferred by means of a sterile mouth pipette to an agar-lined dish containing the host embryo. A slit was prepared in the host epidermis in the dorsal region just anterior to the dorsal fin region and the graft was quickly inserted. Stage 10 donor tissue containing ectoderm, mesoderm and neural tube gave rise to both micro- and macromelanophores; when neural tube was lacking, pigment was not produced. At a later stage, ectoderm and mesoderm gave rise to pigment cells in grafts.

anophores appeared in the host at the site of the graft, these cases were regarded as positive.

2. If, after the host melanophores had populated the graft area, pigment cells appeared *inside* the graft and upon sectioning could be shown to be within the graft, these also were regarded as of donor origin.

3. Any graft which became populated with melanophores at about the same time the host cells migrated into the grafted region was considered negative, since by observation it was not possible to distinguish between host and donor micromelanophores.

1. *Grafts Containing Neural Tissue.*—In the first series of operations, totalling 25 animals, the tissue obtained from the region of the first somite of the hybrid donor embryo at Stage 10 contained ectoderm, somite mesoderm and part of the nerve cord (see Table 1). In 17 out of 25 swordtail hosts, micromelanophores were present in the graft, with one case in doubt. The seven remaining cases were regarded as negative although these, too, may actually represent grafts which differentiated their hybrid donor melanophores too late to be clearly distinguished from host pigment cells.

In addition to pigment cells, the grafted tissue in this series was found to contain well differentiated muscle and nerve tissue. In some of the embryos that were permitted to develop for a longer period of time, cartilagenous skeletal elements were found, Plate II, Fig. 4.

2. *Grafts without Nervous Tissue.*—In the second series of 14 grafted embryos, the donor tissue from a platyfish-swordtail hybrid was taken with particular care to exclude nervous tissue. This was done with iridectomy scissors by first removing from a Stage 10 donor a piece of tissue that usually contained ectoderm, somite mesoderm and neural tissue. The neural tissue was then carefully dissected away by means of glass needles and discarded. The remaining piece was trimmed to an appropriate size and implanted in a swordtail host embryo. Of the 14 experiments done in this way, 3 host embryos gave rise to micromelanophores of donor origin. In 11 host embryos, no donor pigment cells appeared before host pigment cells had surrounded and covered the graft area.

3. *Time of Migration.*—The last series of experiments was carried out using tissue from platyfish-swordtail embryo donors at Stage 13. Grafts containing ectoderm and mesoderm were placed dorsally in Stage 20 swordtail embryo hosts. Micromelanophores appeared in the graft in 7 of the 8 swordtail embryos, while one embryo failed to produce pigment cells. These experiments suggest that during the period between Stage 10 and Stage 13 (from 3½ to 7 days; or from the 13th somite to the 25th somite stage), the pro-pigment cells have migrated from the point of origin in the nervous system and have moved into the more superficial tissues.

Discussion

The evidence presented in this paper confirms the observations of Lopashov (1944), who found that in three species of teleost fishes black pigment cells appeared in the host after pieces of brain were grafted under the epithelium of the yolk sac. When grafts of ectoderm and mesoderm, with and without neural tube, from platyfish-swordtail embryos in comparable stages of development to those used by Lopashov, were

Table 1. Appearance of Pigment Cells in Transplants of Various Tissues from Swordtail-Platyfish Hybrid Embryos into Swordtail Embryos[1]

Donor Stage	Donor Tissue	Number of Cases	Pigment Cells Found in Graft in Host[2]	No Pigment Cells in Graft in Host[2]
10	Neural Tube Ectoderm Mesoderm	25	17 1 doubtful	7
10	Ectoderm Mesoderm	14	3	11
13	Ectoderm Mesoderm	8	7	1

[1] For criteria see text, pages 4 and 6.
[2] Swordtail embryo Stage 22.

implanted into swordtail embryos, good evidence was found that the presence of neural tissue is required for the differentiation of black pigment cells. Lopashov was also able to show that the introduction of mesoderm alone in most cases did not lead to the production of pigment cells. Although one series of experiments reported in this paper indicated that pro-pigment cells were present in the mesoderm, this was undoubtedly due to the fact that the donors used for these grafts were somewhat older than those used for the other experiments, or those used by Lopashov. Accordingly, it was concluded that between Tavolga Stages 10 and 13, the migration of pro-pigment cells probably occurs in embryonic xiphophorin fishes. Further experiments are in progress to check this point.

Of perhaps more interest than the appearance of pigment cells from the neural tissue grafts were the types of pigment cells that made their appearance. Micromelanophores were abundant in the graft, which is in keeping with the picture in the normal fish. The presence of macromelanophores in the tissue graft is of particular interest. Since the normal experimental swordtail host embryos never produce macromelanophores, the only possible source of these cells is from the donor tissue of potentially melanomatous platyfish-swordtail hybrids. The donor embryos from which these cells were taken were at Stage 10 or younger, and the tissue taken included nerve cord, somite mesoderm and ectoderm. If the evidence of Lopashov concerning the non-appearance of melanocytes from purely mesodermal grafts may be assumed to hold in these forms, it seems likely that the macromelanophores arose from the same region of the nervous system as the micromelanophores. This region of the nervous system of the fish embryo is presumably homologous with the neural crest in higher forms.

The relative infrequency of macromelanophores in our experimental embryos is due to two factors. First, the expected incidence of the larger pigment cells in the grafts made was only 50% because only 50% of the backcross platyfish-swordtail embryo donors carried *Sd* gene for macromelanophores. Second, the larger black pigment cells normally appear in genotypically *Sd* fish only postnatally; rarely do they appear just prior to birth. Therefore, the macromelanophores in the grafts observed in the swordtail embryo hosts would not represent the entire possible population of these cells, but rather only those that had made their appearance at the time of death, by fixation, of the host.

It has been an open question as to whether melanoma cells are of primary (embryological) or secondary (derived) origin. It was previously impossible to determine by direct observation whether definitive melanoma cells, particularly the macromelanophores, were embryologically-derived normal body constituents which in their ontogeny become malignant in response to their genotype, or whether these and other pigment-containing cells in the melanoma had become pigmented and malignant as a result of influences external to the cells. In the platyfish-swordtail hybrid the theoretical problem of true pigment cell origin can be resolved because it now appears that macromelanophores, at least, are normal products of the embryonic nervous system. To complete the picture, Gordon (1937) has shown that macromelanophores, in their development in hybrids of appropriate genotype, initiate the growth of melanomas. Marcus & Gordon (1954) and Ermin & Gordon (1955) presented evidence that some melanocytes, which are melanin-synthesizing cells, transform into macromelanophores; pigment-containing hypertrophic fibroblasts, giant cells and connective tissue stroma cells, however, are secondary pigmented cells that acquire their melanin from contact with macromelanophores and melanocytes.

The neural theory of melanoma development in hybrid xiphophorin fish seems to be supported by direct evidence derived from the application of genetic and experimental embryological techniques. The extension of this type of experimental approach to the problem of the embryological origin of mammalian melanomas has as yet not been achieved. Therefore the conclusions that may be drawn concerning the origin of mammalian melanomas must be tentative. However, the observations that pigmentation in the mouse originates from cells derived from the neural crest (Rawles, 1953) and the morphological resemblance of the primary pigment cell types (melanocytes) found in fish and mouse and human melanomas (Grand, Gordon & Cameron, 1941) argues in favor of a similarity in the final tumors and in their etiology.

Summary

The origin of pigment cells in xiphophorin fish was studied by grafting tissue from platyfish-swordtail hybrid embryos (50% of which were genetically destined to develop a melanosis or a melanoma) into wild-type swordtail embryos.

When the grafts from a Stage 10 hybrid donor embryo contained ectoderm, mesoderm and nervous tissue they gave rise in the swordtail host embryo first to micromelanophores, and later to macromelanophores, as well as to muscle, nervous tissue, and in some cases to cartilagenous elements.

When the grafted tissue from Stage 10 hybrid

donors contained only ectoderm and somite mesoderm, micromelanophores appeared in only three of the 14 swordtail host embryos prior to the appearance of host pigmentation; macromelanophores appeared in none.

Using only ectoderm and somite mesoderm tissue grafts from a Stage 13 hybrid donor, pigment cells appeared in 7 out of 8 swordtail embryo hosts; this indicated that pro-pigment cells migrate from the nervous system to the more superficial layers of the hybrid embryo between Stages 10 and 13.

The presence of macromelanophores in the grafted tissue, and therefore of donor nervous system origin, permits a re-evaluation of the neural crest theory of melanoma cell origin. From the evidence presented in this paper, it seems likely that the genetically determined macromelanophores that give rise to the melanomas of platyfish-swordtail hybrids are normal derivatives of the nervous system. This in part confirms the conclusion reached by geneticists that heredity is primarily responsible for the growth of melanomas in these animals.

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EXPLANATION OF THE PLATES

PLATE I

FIG. 1. Swordtail host embryo 24 hours after the implantation of a graft (containing ectoderm, mesoderm and neural tube from a stage 10 hybrid donor) into a notch just anterior to the dorsal fin region. The graft appears as a small lump. × 50

FIG. 2. The same host 3 weeks after grafting. Note the considerable growth and differentiation of the graft and the appearance of a macromelanophore at its base. × 20

PLATE II

FIG. 3. Cross-section of transplanted ectoderm, mesoderm and neural tube. Transplant taken from a Stage 10 platyfish-swordtail hybrid donor and placed in a swordtail host at Stage 20. Host killed after 10 days. mm = macromelanophore.

FIG. 4. Section as above, showing differentiated muscle (m); macromelanophores (mm); nervous tissue (n); and cartilage (c) of graft origin.